FORM PTO-1390 (REV 10-2000)

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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: UF-219XC1

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INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/11940	May 28, 1999	May 29, 1998

TITLE OF INVENTION: Combination Therapy for Treatment of FIV Infection

APPLICANT(S) FOR DO/EO/US: Ben M. Dunn, Janet K. Yamamoto, Maki Arai

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [x] This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. [x] The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. [x] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [x] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Ofice (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19(35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C371(c)(3)).
9. [x] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unexecuted)
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/763037	PCT/US99/11940	UF-219XC1

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

	CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	15 - 20 =	0	X **$18.00**	$ 0.00	
Independent claims	3 - 3 =	0	X **$80.00**	$ 0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 860.00	
[X] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$ 430.00	
SUBTOTAL =				$ 430.00	
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TOTAL NATIONAL FEE =				$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 0.00	
TOTAL FEES ENCLOSED =				$ 430.00	
				Amount to be refunded:	$
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a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 19-0065 in the amount of $ 430.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pendin g status.

SEND ALL CORRESPONDENCE TO:

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38,261
REGISTRATION NUMBER

DESCRIPTION

COMBINATION THERAPY FOR TREATMENT OF FIV INFECTION

The subject invention was made with government support under a research project supported by NIH Grant AI30904. The government has certain rights in this invention.

Background of the Invention

Feline immunodeficiency virus (FIV) is a lentivirus which causes immunodeficiency syndrome in domestic cats (Pedersen *et al.*, 1987; Siebelink *et al.*, 1990). FIV closely resembles human immunodeficiency virus (HIV) in genomic, biochemical, and morphologic characteristics as well as clinical and hematological manifestations (Johnson *et al.*, 1994; Pedersen *et al.*, 1987; Yamamoto, Sparger *et al.*, 1988). As a result, FIV infection of domestic cats is considered to be an excellent small animal model for testing prophylactic and therapeutic strategies against AIDS viruses (Gardner, 1991; Johnson *et al.*, 1994). A number of antiretroviral drugs for HIV, including the prototype nucleoside analogue azidothymidine (AZT), has been tested using the FIV model (Hart *et al.*, 1995; Hartmaun *et al.*, 1992; Hayees *et al.*, 1993; Hayees *et al.*, 1995; Meers *et al.*, 1993; North *et al.*, 1989; Smith *et al.*, 1994).

The therapeutic use of AZT has been unremarkable in cats and was unable to delay the spread of FIV infection *in vivo* (Hart *et al.*, 1995; Hartmaun *et al.*, 1992). Prophylactic AZT treatment of experimental cats caused either a delay or decrease in both infected blood lymphocyte numbers and plasma virus load (Hayees *et al*, 1993; Hayees *et al.*, 1995; Meers *et al.*, 1993; Smith *et al.*, 1994). In addition, a delay in FIV antibody production was observed in some animals (Smith *et al.*, 1994). However, prophylactic therapy with AZT did not protect cats from FIV infection (Meers *et al.*, 1993; Hayees *et al.*, 1993; Hayees *et al.*, 1995; Smith *et al.*, 1994). As reported for HIV therapy, withdrawal of the drug resulted in a resurgence of the virus in these cats. When compared to the untreated group, significantly higher CD4 and CD8 cell counts were observed shortly after the withdrawal of the drug (Hayees *et al.*, 1993; Hayees *et al.*,

1995). However, CD4/CD8 ratios were not significantly different from the untreated cats. In contrast, FIV-infected cats therapeutically treated with AZT had no change in FIV antigen or anti-FIV antibody titers but had transient improvement in CD4/CD8 ratios and clinical signs (Hart *et al.*, 1995; Hartmaun *et al.*, 1992). These findings suggest that monotherapy with AZT has limited benefit as a therapy for FIV infection. Similar observations have been made with AZT monotherapy of HIV-infected individuals (Harrigan, 1995; Staszewski, 1995).

In recent trials, combination therapies with AZT and other antiretroviral drugs, such as phosphonomethoxyethl) adenine and dideoxycytidine 5'-triphosphate, had minimal to no effect in preventing or controlling FIV infection in cats (Hartmaun *et al.*, 1992; Magnani *et al.*, 1994; Philpott *et al.*, 1992). The *in vivo* use of viral protease inhibitors or new nucleoside analogue combinations, such as, for example, lamivudine (3TC) and AZT has yet to be reported in FIV-infected cats. Commercially available HIV protease inhibitors (*e.g.*, Sequinavir (SQV), Indinavir (IDV), Ritonavir, Nelfinavir) do not inhibit FIV replication in PBMC *in vitro*. Unlike other nucleoside analogues, 3TC rapidly induces mutations which can phenotypically reverse the mutations caused by AZT, enabling the antiviral activity of AZT to persist in the host (Boucher *et al.*, 1993; Larder, 1995; Tisdale *et al.*, 1993). This unique feature of 3TC makes it a prime candidate for use in combination with AZT. In HIV-positive individuals, the combination AZT/3TC therapy had synergistic or additive effects at decreasing plasma virus load and increasing CD4 cell counts and function (Katlama *et al.*, 1994; Lange, 1995; Paul *et al.*, 1995; Staszewski, 1995). The addition of an HIV protease inhibitor to this combination further decreased the viral load and improved the CD4 cell count (Deeks *et al.*, 1997; Torres *et al.*, 1997).

Brief Summary of the Invention

The subject invention concerns methods for therapeutic and prophylactic treatment of feline animals against infection by FIV. Methods of the present invention utilize a combination of antiretroviral compounds. In one embodiment, an effective amount of a composition comprising AZT and another nucleoside such as 3TC . In another embodiment, cats are given an effective dose(s) of a composition comprising

AZT, a nucleoside analog such as 3TC and a retroviral protease inhibitor. In an exemplified embodiment, the protease inhibitor is HBY-793 (Hoescht-Bayer).

Brief Description of the Drawings

Figure 1 shows anti-FIV activities of AZT, 3TC, FIV-PI, and HIV-PI (IDV and SQV) in chronically FIV-infected cell lines. The antiviral activity of the drugs at noncytotoxic doses were evaluated in feline T-cell lines chronically infected with either FIV_{Pet} (subtype A strain) (panel A), or FIV_{Bang} (subtype B strain) (panel B). The RT data are presented as % control, whereby % control represents RT mean of triplicate treated cultures divided by RT mean of triplicate untreated cultures and multiplied by 100. The RT data on harvest days at 6, 9, and 12 are shown. The results from treated culture sets which are statistically different from the values of the untreated controls are indicated by either $p<0.05$ *(P)* or $p<0.005$ (*P**) based on Student T test.

Figure 2 shows anti-FIV activities of AZT, 3TC, FIV-PI, and FIV-PI in primary PBMC infected with FIV_{Bang}. Six separate experiments with varying concentrations and combinations were performed and the results from two representative experiments are shown. Nucleoside analogue and PI doses were 0.1 μM in Experiment 1 (panel A) and 0.05 μM and 0.01 μM, respectively, in Experiment 2 (panel B). The RT data are presented as % control and the results from treated culture sets which are statistically different from the values of the untreated controls are indicated by either $p<0.05$ (*P*) or $p<0.005$ (*P**) based on Student T test. The Harvest Day 16 result for AZT/3TC culture set was statistically different ($p<0.03$) from the results of AZT culture set and 3TC culture set from the same time point, as indicated by (Y) above AZT/3TC bar (panel A). The Day 9 and 12 harvest results for AZT/3TC/FIV-PI culture set were statistically different ($p<0.05$) from the results of AZT/3TC culture set and FIV-PI culture set from the same time points, as indicated by (*Z*) above AZT/3TC/FIV-PI bars (panel B).

Figure 3 shows anti-FIV activities of AZT, 3TC, FIV-PI, and HIV-PI in primary PBMC infected with FIV_{UK-8} (subtype A strain). Four separate experiments with varying concentrations and combinations were performed and the results from two representative experiments are shown. Nucleoside analogue and P1 doses were 0.1 μM and 0.01-0.5 μM, respectively, in Experiment 1 (panel A) and 0.05 μM and 0.01-0.5 μM respectively, in Experiment 2 (panel B). The RT data are presented as % control and the results from

treated culture sets which are statistically different from the values of the untreated controls are indicated by either $p<0.05$ (*P*) or $p<0.005$ (*P**). Statistical differences existed between the results of AZT/3TC culture set and 3TC culture set at Harvest Days 17 ($p<0.02$) and 20 ($p<0.001$) in panel A and Harvest Days 9 ($p<0.02$), 12 ($p<0.04$), and 15 ($p<0.02$) in panel B, as indicated by (X) above the AZT/3TC bars. In addition, statistical difference existed between the results of AZT/3TC culture set and AZT culture set at Harvest Day 20 ($p<0.001$) in panel A and Harvest Day 15 ($p<0.01$) in panel B, as indicated by (*Z*) above the AZT/3TC bars.

Figure 4 shows the chemical structure of the protease inhibitor designated herein as HBY-793.

Detailed Disclosure of the Invention

The subject invention concerns methods for therapeutic and prophylactic treatment of cats against infection by FIV. Methods of the present invention utilize a combination of antiretroviral compounds. In one embodiment, cats can be administered an effective amount of a composition comprising AZT and another nucleoside analog. Preferably, the nucleoside analog is 3TC.

In another embodiment of the methods of the present invention, cats are given an effective dose(s) of a composition comprising AZT, another nucleoside analog and a retroviral protease inhibitor. Preferably, the nucleoside analog is 3TC. In an exemplified embodiment, the protease inhibitor is HBY-793. The structure of HYB-793 is shown in Figure 4. Other retroviral protease inhibitors that can inhibit FIV proteases are contemplated within the scope of this invention.

FIV-infected cats treated according to the methods of the present invention can also be given bone marrow transplantation after total body irradiation in conjunction with the antiretroviral drug combination therapy. The bone marrow transplanted can be either allogeneic or autologous.

The antiretroviral compositions of the subject invention can be administered using standard procedures known in the art. For example, the compositions can be administered as oral or nasal formulations. The compositions can also be administered by parenteral injection, *i.e.*, intravenous, intramuscular, or subcutaneous injection. The

amounts and dosage regimens for administration can readily be determined by the ordinarily skilled clinician.

Cats that are not infected with FIV can be treated according to the methods of the present invention to provide effective prophylactic treatment against FIV infection. FIV-infected cats can be treated according to the subject methods to provide effective therapy for controlling, inhibiting or eliminating FIV infection in that cat.

Results from studies described herein show that the addition of a nucleoside analog like 3TC to prophylactic AZT therapy will completely protect cats against FIV infection. This observation is supported by the *in vitro* findings demonstrating that an AZT/3TC combination was more effective at inhibiting FIV replication in PBMC cultures than single-drug treatments using AZT or 3TC alone. The AZT/3TC combination is effective when used prophylactically or immediately upon FIV exposure. In addition, the combination of antiretroviral drugs AZT/3TC/FIV-PI can be used as an anti-FIV therapy to treat chronically infected animals.

The present invention also concerns kits comprising in one or more containers AZT, another nucleoside analog and an inhibitor of a retroviral protease. Preferably, the nucleoside analog is 3TC. In a preferred embodiment, the retroviral protease inhibitor is HBY-793.

The following abbreviations of FIV strains are used herein:

Strain (subtype)	Abbreviation
Petaluma (A)	FIV_{Pet}
Dixon (A)	FIV_{Dix}
UK8 (A)	FIV_{UK-8}
Bangston (B)	FIV_{Bang}
Aomori-1 (B)	FIV_{Aom1}
Aomori-2 (B)	FIV_{Aom2}
Shizuoka (D)	FIV_{Shi}

All references cited herein are incorporated by reference.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – *In vitro* Efficacy of AZT, 3TC, and PI

In the first set of *in vitro* studies, feline T-cell lines chronically infected with FIV_{Pet} (FL-4 cells) or FIV_{Bang} (FIV_{Bang}/FeT-J cells) at $2x10^5$ cells/ml were treated for 3 weeks with a single drug or various combinations of AZT, 3TC, an FIV protease inhibitor (FIV-PI; Hoescht-Bayer HBY-793), and HIV protease inhibitors (HIV-PI) (Fig. 1A and 1B). Saquinavir (SQV) and Indinavir (IDV) were used as the HIV-PIs. Culture supernatants were harvested and the cells were resuspended in fresh culture media containing appropriate drug(s) at 34 day intervals. Viral replication was determined by measuring the levels of reverse transcriptase (RT) activity in the culture supernatants (Rey *et al.*, 1984). Drug toxicity in these cultures were monitored by viability and absolute cell count analyses using trypan blue exclusion method (Mishell *et al.*, 1980). Single and combination drug doses which were determined to be nontoxic to the test cells were used in these studies.

Both single and combination treatments with AZT and 3TC had minimal to no effect at inhibiting RT activity in FIV_{Bang}/FeT-J cells (20-50% inhibition) and FL-4 cells (0-10% inhibition). In contrast, FIV-PI treatment inhibited FIV replication by 70-80% in both cell lines (Fig. 1A and 1B). However, the addition of an AZT/3TC combination did not enhance this inhibition. Furthermore, neither SQV nor IDV alone had significant anti-FIV effect (Fig. 1A and 1B). The differences in anti-FIV activities of these nucleoside analogues and FIV-PI may be due to the differences in the mechanism(s) of their antiviral activities. AZT and 3TC exert their antiretroviral activity by preventing the reverse transcription of viral RNA into viral DNA, whereas FIV-PI prevents the production of a whole virion by inhibiting the FIV protease from cleaving viral gag-pro--pol precursor into their individual components. Therefore, cell lines which have proviral integration will not be affected by nucleoside analogues. Based on semi-quantitative PCR analysis, FIV_{Bang}/FeT-J cells and FL-4 cells used in current study had proviral integration of 50-80% and >95%, respectively (data not shown). The minor anti-FIV activity of AZT and 3TC observed in FIV_{Bang}/FeT-J cells may be due to the antiviral

effect of the nucleoside analogues on the 20-50% of the cells which were still free of FIV proviral integration. As expected, potent anti-FIV activity was observed with FIV-PI in both proviral integrated cell lines.

As a means to simulate *in vivo* conditions, primary peripheral blood mononuclear cells (PBMC) from specific pathogen free (SPF) cats were next used as the indicator cells. Primary PBMC isolated by ficoll hypaque method were stimulated with concanavalin A for 3 days and cultured for an additional 2 weeks before their use in drug studies (Staszewski, 1995). Antiretroviral drug(s) were added to the PBMC cultures ($1x10^6$ cells/ml) immediately before FIV_{Bang} (subtype B) or FIV_{UK-8} (subtype A) inoculation of 100 50% tissue culture infectious dose ($TCID_{50}$). Both single and combination treatments with AZT and 3TC inhibited the FIV replication in PBMC at doses which were not toxic to the cells (Fig. 2A and 3A). Synergism in antiviral activities of AZT/3TC combination was observed against both FIV_{Bang} and FIV_{UK-8} strains (Fig. 2A, 3A, and 3B). The addition of the FIV-PI to the AZT/3TC combination further enhanced the activities of these drugs against FIV_{Bang} (Fig. 2B). Such enhancement was not observed against FIV_{UK-8} at the doses used (Fig. 3A and 3B). Thus, the anti-FIV activities of AZT, 3TC, and FIV-PI are not restricted to specific FIV strain or subtype, although some strains appear to be more sensitive to one drug over another. Similar to previous studies with chronically infected cells, single-drug treatments with FIV-PI but not HIV-PIs (SQV and IDV) inhibited FIV replication in PBMC cultures (Fig. 2A, 3A, and 3B). Furthermore, addition of SQV or IDV to the AZT/3TC combination did not enhance the antiviral activity of the AZT/3TC combination. The lack of anti-FIV activity of SQV and IDV may be explained by the fact that HIV-PIs do not efficiently bind to FIV protease, whereas the FIV-PI used in this study efficiently binds to HIV protease as well as FIV protease (Dunn *et al.*, 1994; Wlodawer *et al.*, 1995). These results show that dual and triple combinations of AZT, 3TC, and FIV-PI may have therapeutic benefit against FIV infection in domestic cats.

Example 2 – Prophylactic Efficacy of AZT/3TC in Cats

Based on the findings from *in vitro* studies, the prophylactic use of AZT/3TC combination was next tested in experimental cats. Four of the eight SPF cats (16-20 weeks of age) received oral administration of AZT and 3TC (75 mg/kg each) twice a day

(BID), while remaining cats received placebo. This treatment dose was based on the *in vivo* research, in which six SPF cats (2 cats per treatment group) treated (BID) with either AZT or 3TC at 100 mg/kg or AZT/3TC combination at 50 mg/kg each had no hematological or clinical abnormalities after two weeks of treatment. In this study, all cats except for one treated cat (#RUl) were inoculated with 100 50% cat infectious dose ($C1D_{50}$) of FIV_{UK-8} at 3 days after the first drug or placebo treatment. FIV_{UK-8} was used in this study because this strain gave more consistent CD4/CD8 ratio inversion in a larger number of infected cats than did infection with FIV_{Bang} or FIV_{Pet}. All cats received either the drug or placebo treatments throughout the first 11 weeks after FIV inoculation, unless stated otherwise. The cats were monitored daily for clinical signs and twice a month for hematological changes, FIV load in PBMC and plasma, anti-FIV antibody titers, and CD4/CD8 ratio and absolute counts (Diehi *et al.*, 1995; Green *et al.*, 1993; Okada *et al.*, 1994; Tellier *et al.*, 1997; Yamamoto *et al.*, 1991).

At 4 weeks of treatment, severe anemia was observed in all challenged and unchallenged cats treated with AZT/3TC; therefore, the doses of each drug were lowered to 34 mg/kg each at 4 weeks of treatment and subsequently to 5-10 mg/kg each at 5 weeks of treatment. AZT/3TC treatment was terminated in one cat (#3GB) at 6 weeks of treatment, and the treatment was resumed 6 days later at 5 mg/kg each. Based on virus isolation and PCR analyses, one cat (#101) from the placebo group was positive for FIV by 3 weeks post infection (pi) and had anti-FIV antibodies by 5 weeks pi (Table 1). However, plasma viral RNA levels of this cat were not detected throughout the study; even though the virus load in the PBMC was similar to the levels detected in the remaining placebo cats. These placebo cats (#NK4, #NK6, #IH5) were positive for FIV titers in the plasma and PBMC and for anti-FIV antibodies by 7 weeks pi. Furthermore, all placebo cats, except for cat #101, had transient or persistent CD4/CD8 inversion starting 11 weeks pi. In contrast, all AZT/3TC-treated cats were negative for FIV and had no CD4/CD8 inversion throughout the study. Both drug and placebo treatments were terminated at 11 weeks pi and all cats were monitored for additional 6-13 weeks. In the previous reports, an increase in FIV load of the PBMC was observed after the withdrawal of AZT treatment in FIV-infected cats (Hayees, *et al.*, 1993; Hayees *et al.*, 1995; Meers *et al.*, 1993). Thus, if low levels of FIV infection undetectable by current assays existed in AZT/3TC-treated cats, then such infection should rebound when the drugs are

removed. In this study, all AZT/3TC-treated cats remained negative for FIV in PBMC and anti-FIV antibodies throughout the 6-13 weeks after the withdrawal of the drug treatment. Virus isolation and PCR of bone marrow and lymph node cells performed at the termination of the study further confirmed the FIV-free status of these cats. Thus, complete protection of cats against experimental FIV infection was achieved with prophylactic AZT/3TC therapy.

Example 3 – Therapeutic Efficacy of AZT/3TC in Chronically FIV-Infected Cats

Based on the *in vivo* toxicity observed in the prophylactic study, three cats (#101, #NK6, #144) chronically infected with FIV_{UK-8} for 16 weeks were treated at 20 mg/kg of each drug (BID), while an additional three infected cats (#1H5, #NK4, #158) received placebo. These cats were treated with either drug combination or placebo for 8 weeks and monitored an additional 4 weeks for changes in FIV load and CD4/CD8 values. All parameters monitored were identical to those of the prophylactic study. All treated cats developed either mild or severe anemia by 3.5 weeks of treatment. As a result, both drug doses were lowered to 10 mg/kg. Nevertheless, the anemia in one cat (#144) became so severe by 6 weeks of treatment that the drug treatment was terminated for 1 week and resumed thereafter at a low dose of 5 mg/kg of each drug (BID). Unlike the prophylactic study, no significant differences in either FIV load or CD4/CD8 ratios and absolute counts were observed between the treated and placebo cats (Table 2). This study in combination with the previous studies suggest that doses even as low as 20 mg/kg of each drugs when used over moderate period of time (3.5 weeks or longer) will cause anemia in cats. However, short-term treatment (2 weeks) with high dose combination (75 mg/kg each) is well tolerated by cats.

Example 4

Allogeneic bone marrow transplantation (BMT) in combination with total body irradiation (TBI) and anti-FIV drug therapy was evaluated as an immune reconstitution therapy for FIV-infected cats. The rationale for this therapy is as follows: (1) TBI will decrease FIV load by destroying recipient's hematopoietic cells, including FIV-infected immunocytes. (2) Anti-FIV drug therapy can block the infection of engrafted donor cells in the BMT recipients. (3) BMT with donor BM cells from uninfected cats will

reconstitute normal hematopoietic system. The TBI/BMT combination alone was unable to decrease the virus load due to rapid infection of engrafted donor cells. A majority of FIV-infected recipients of allogeneic BMT succumbed to graft-versus-host disease, accelerated FIV-related diseases, or their combination. As a result, studies were performed to identify antiretroviral drugs that can be combined with TBI/BMT. Prophylactic therapy with AZT/3TC combination protected 100% of the cats from FIV_{UK-8} challenge infection. Moreover, the only FIV-infected cat to survive allogeneic BMT also received concurrent AZT/3TC therapy. This cat had complete hematopoietic engraftment including normal CD8 counts. However, its CD4 counts were only slightly higher than the levels observed before BMT. Furthermore, only slight decrease in plasma virus load was observed during high-dose AZT/3TC therapy. Nonetheless, its anti-FIV antibody titers were 100-fold lower than those before BMT. This cat was still healthy at one year post-BMT and is still responsive to AZT/3TC therapy.

Example 5

Recent findings with anti-HIV triple-drug combination have revealed that triple-drug cocktails are unable to immune reconstitute the patient with normal numbers and repertoire of T cell populations or to completely decrease/remove the virus load in the lymphoid tissues within feasible duration of time. As such, autologous bone marrow transplantation (BMT) was tested in combination with antiretroviral drugs as an immune reconstitution therapy for FIV-infected cats. Based on preliminary results, no significant decrease in FIV load or improvement in CD4/CD8 ratios or counts were detected in infected cats that received autologous BMT one (1) day after total body irradiation (TBI). These cats survived the autologous BMT and are currently alive over two years after BMT. This is in contrast to the results from allogeneic BMT of FIV-infected cats, whereby all cats except the one on AZT/3TC therapy succumbed to GVHD, accelerated FIV-disease, or their combination. The extension of the time of BMT after TBI will decrease the infected cell reservoir load and, consequently, fewer infected cells will be available to infect engrafted cells. Addition of antiretroviral drug therapy will prevent any remaining infected cell reservoir from contaminating the engrafted cells.

Example 6 – Pharmaceutical Compositions

Antiviral compounds of the invention can be formulated according to known methods for preparing pharmaceutically useful compositions. *Remington's Pharmaceutical Science* by E.W. Martin describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the antiviral compounds are is combined with a suitable carrier in order to facilitate effective administration of the composition. It should, of course, be understood that the compositions and methods of this invention may be used in combination with other therapies.

The compositions used in these therapies may also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspensions, liposomes, suppositories, injectable, and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and adjuvants which are known to those of skill in the art. Preferably, the compositions of the invention are in the form of a unit dose.

Once improvement in condition has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

TABLE 1. AZT/3TC prophylaxis of cats starting 3 days before FIV inoculation

	AZT/3TC treatment[a]		FIV levels[b]													
	(kg/mg)	FIV	VI/PCR/vRNA					FIV antibodies[b]					CD4/CD8 ratio[bc]			
Cat #	-0.4→4→5→6-7 wk	inocul.	Pre	4 wk	9 wk	11 wk	14 wk	Pre	4 wk	9 wk	11 wk	14 wk	Pre	7 wk	11 wk	14 wk
DH5	75→34→10	+	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-	-	-	-	-	3.30	2.86	2.62	2.38
3GB	75→34→10→0-5	+	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-	-	-	-	-	1.56	1.37	1.37	1.47
RU2	75→34→10	+	-/-/-	-/-/-	-/-/-	-/-/-	-/-	-	-	-	-	-	1.77	1.21	1.18	1.18
RU1	75→34→10	-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-	-	-	-	-	2.37	1.62	1.62	1.47
NK4	-	+	-/-/-	+/+/+	+/+/+	+/+/+	+/+/+	-	-	+	+	+	1.82	1.55	0.96	0.91
NK6	-	+	-/-/-	-/+/+	+/+/+	+/+/+	+/+/+	-	-	+	+	+	1.61	0.92	0.46	0.45
1O1	-	+	-/-/-	+/+/-	+/+/-	+/+/-	+/+/-	-	-	+	+	+	3.40	1.73	1.24	1.23
IH5	-	+	-/-/-	-/+/+	+/+/+	+/+/+	+/+/+	-	-	+	+	+	4.40	1.34	0.60	0.61

[a] The AZT/3TC treatment was started 3 days before FIV inoculation (-0.4 post-infection) at a dose of 75 mg/kg each and decreased to 34 mg/kg at 4 wk post-infection (pi) and then to 10 mg/kg at 5 wk pi. In one cat (#3GB), the AZT/3TC treatment was withdrawn at 6 wk pi and resumed at a low dose of 5 mg/kg at 7 wk pi. The changes in doses if each drug, including the amount (mg/kg) and time (wk pi), are shown.

[b] Samples before drug or placebo treatment (Pre) and those at various weeks post-infection (wk) were tested for FIV levels, FIV antibodies, and CD4/CD8 rations. FIV levels were determined by virus isolation (VI), PCR for FIV provirus in PBMC, and RT-PCR for plasma viral RNA (vRNA). FIV antibodies were determined by immunoblot analysis. In general, RT-PCR for plasma viral RNA was less sensitive than PCR of FIV provirus in PBMC after amplification of infected cells by coculturing.

[c] Inverted CD4/CD8 ratios are bolded.

TABLE 2. AZT/3TC therapy of FIV-infected cats

Cat No.	AZT/3TC treatment[a] (kg/mg) 0 wk→3.5 wk→6-7 wk	FIV load[b] (No. of infected cells in PBMC) 0 wk	3.5 wk	8 wk	12 wk	FIV antibodies[b] 0 wk	3.5 wk	8 wk	12 wk	CD4/CD8 ratio[b] 0 wk	3.5 wk	8 wk	12 wk
101	20→→10	++	+	+	++	+	+	++	++	1.45	1.58	1.71	1.71
NK6	20→10	++	++	+	+	++	++	++	++	0.45	1.00	0.64	0.70
144	20→10→0-5	+++	++++	+++	++	++	++	++	++	0.67	0.74	0.45	0.52
IH5	-	++	++	+	ND	++	++	++	++	0.61	0.63	0.73	0.87
NK4	-	++	+	+	+	+	++	++	++	0.91	1.25	1.54	1.54
158	-	+++	+++	+++	++	+	++	++	++	1.08	1.01	1.33	1.06

[a] The doses of each drug were decreased from 20 mg/kg to 10 mg/kg at 3 weeks treatment. Treatment was withdrawn in one cat (#144) at 6 weeks of treatment and resumed one week later at 5 mg/kg. The changes in doses of each drug, including amount (mg/kg) and time (wk after initial treatment), are shown.

[b] Samples before drug or placebo treatments (0 wk) and those at various weeks after initial treatment (wk) were tested for FIV levels, FIV antibodies, and CD4/CD8 ratios. FIV loads were determined by the number of PBMC (50 to $5x10^6$ PBMC cocultured with $5x10^6$ feeder PBMC) needed for positive virus isolation. Virus isolation results are presented as 50 (++++), $5x10^2$ (+++), $5x10^3$ (++), and $5x10^4$ (+) PBMC from treated and untreated cats needed to isolate FIV from a culture containing $5x10^6$ uninfected feeder PBMC. FIV antibody titer is defined as the minimal dilution (in Log_{10}) at which antibodies to FIV major core protein (p26) could be detected. Serial log dilutions of serum (10^{-4} to 10^{-7} dilution) were incubated with immunoblot strip for 2 hrs and processed using the immunoblot method. End point titrations of FIV antibodies are presented as 10^{-5} (+) and 10^{-6} (++).

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Claims

1. A method for treating or preventing infection of feline immunodeficiency virus (FIV) in a feline animal, said method comprising administering to said feline animal an effective amount of azidothymidine (AZT) and another nucleoside analog.

2. The method according to claim 1, wherein said another nucleoside analog is 3TC.

3. The method according to claim 1, wherein said feline animal receives bone marrow transplantation after total body irradiation.

4. The method according to claim 3, wherein the transplanted cells are selected from the group consisting of allogeneic cells and autologous cells.

5. A method for treating or preventing infection of feline immunodeficiency virus (FIV) in a feline animal, said method comprising administering to said feline animal an effective amount of azidothymidine (AZT), another nucleoside analog and an inhibitor of a retroviral protease.

6. The method according to claim 5, wherein said another nucleoside analog is 3TC.

7. The method according to claim 5, wherein said inhibitor of a retroviral protease is selected from the group consisting of HIV protease inhibitors and FIV protease inhibitors.

8. The method accoding to claim 5, wherein said inhibitor of a retroviral protease is designated as HBY-793 and has the structure shown in Figure 4.

9. The method according to claim 5, wherein said another nucleoside analog is 3TC and said inhibitor of a retroviral protease is designated as HBY-793 and has the structure shown in Figure 4.

10. The method according to claim 5, wherein said feline animal receives bone marrow transplantation after total body irradiation.

11. The method according to claim 10, wherein the transplanted cells are selected from the group consisting of allogeneic cells and autologous cells.

12. A kit comprising in one or more containers AZT, another nucleoside analog and an inhibitor of a retroviral protease.

13. The kit according to claim 12, wherein said another nucleoside analog is 3TC.

14. The kit according to claim 12, wherein said inhibitor of a retroviral protease is designated as HBY-793 and has the structure shown in Figure 4.

15. The kit according to claim 12, wherein said another nucleoside analog is 3TC and said inhibitor of a retroviral protease is designated as HBY-793 and has the structure shown in Figure 4.

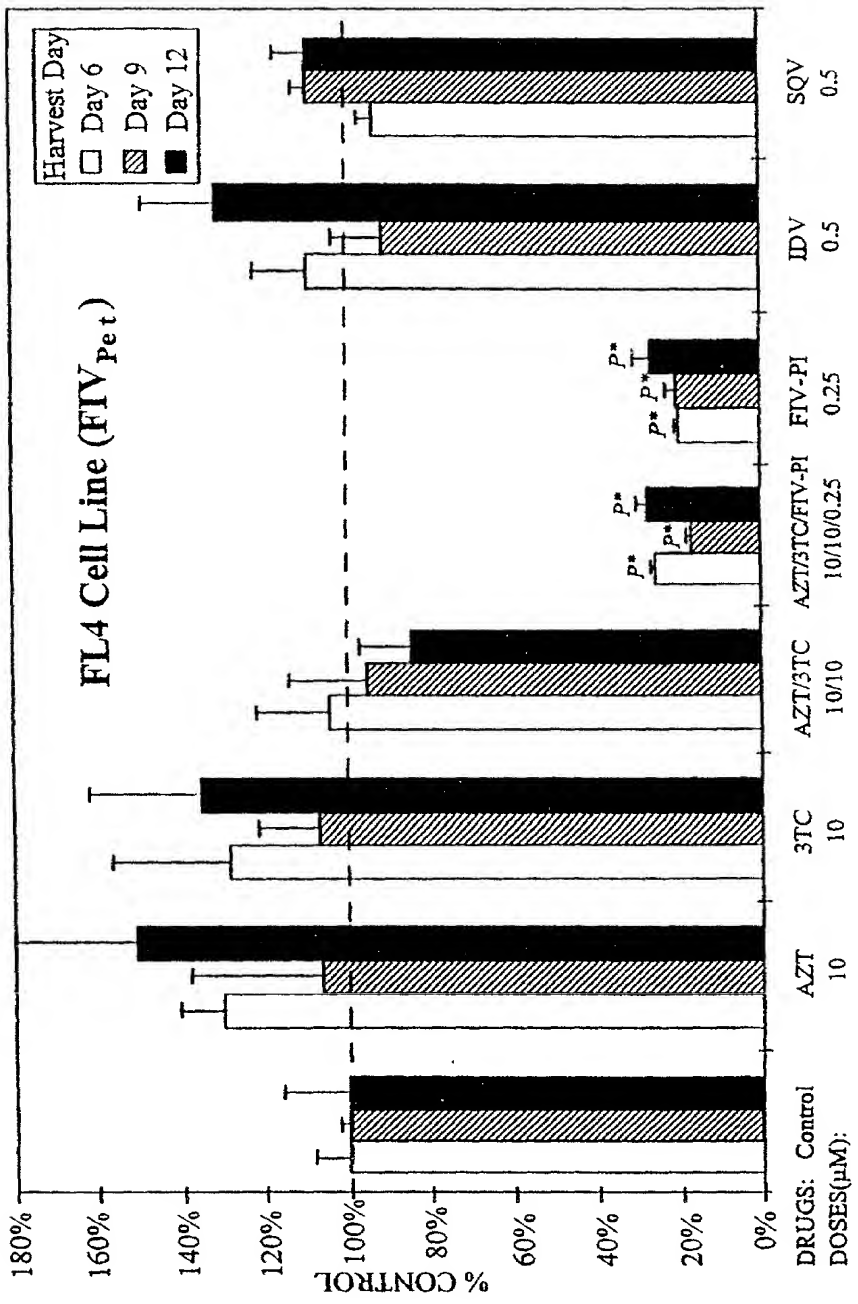
FL4 Cell Line (FIV Pet)
Harvest Day
Day 6
Day 9
Day 12
% CONTROL
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
P*
DRUGS: Control AZT 3TC AZT/3TC AZT/3TC/FIV-PI FIV-PI IDV SQV
DOSES(μM): 10 10 10/10 10/10/0.25 0.25 0.5 0.5

FIG. 1A

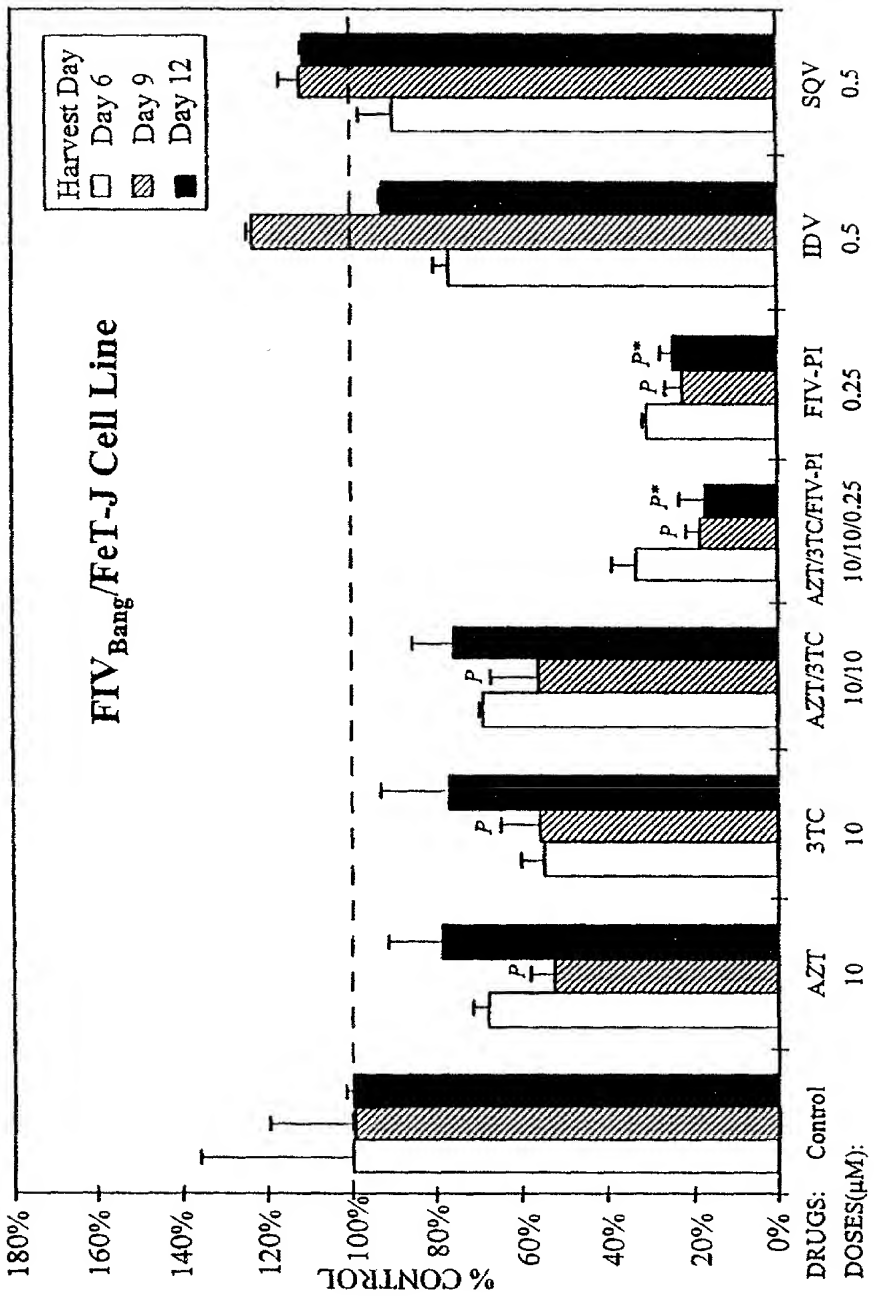
FTV$_{Bang}$/FeT-J Cell Line
Harvest Day
Day 6
Day 9
Day 12
% CONTROL
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
DRUGS: Control AZT 3TC AZT/3TC AZT/3TC/FIV-PI FIV-PI IDV SQV
DOSES(μM): 10 10 10/10 10/10/0.25 0.25 0.5 0.5

FIG. 1B

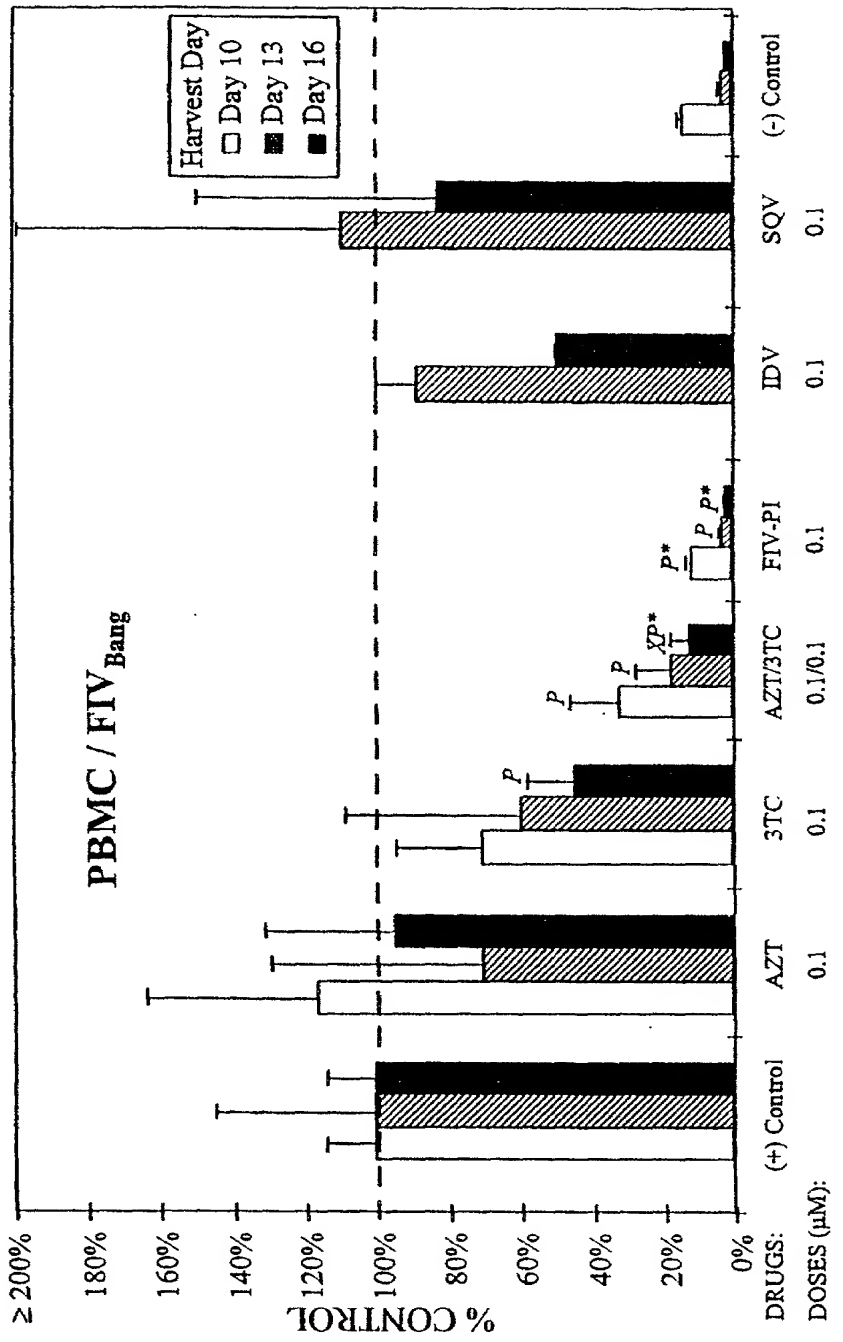

FIG. 2A

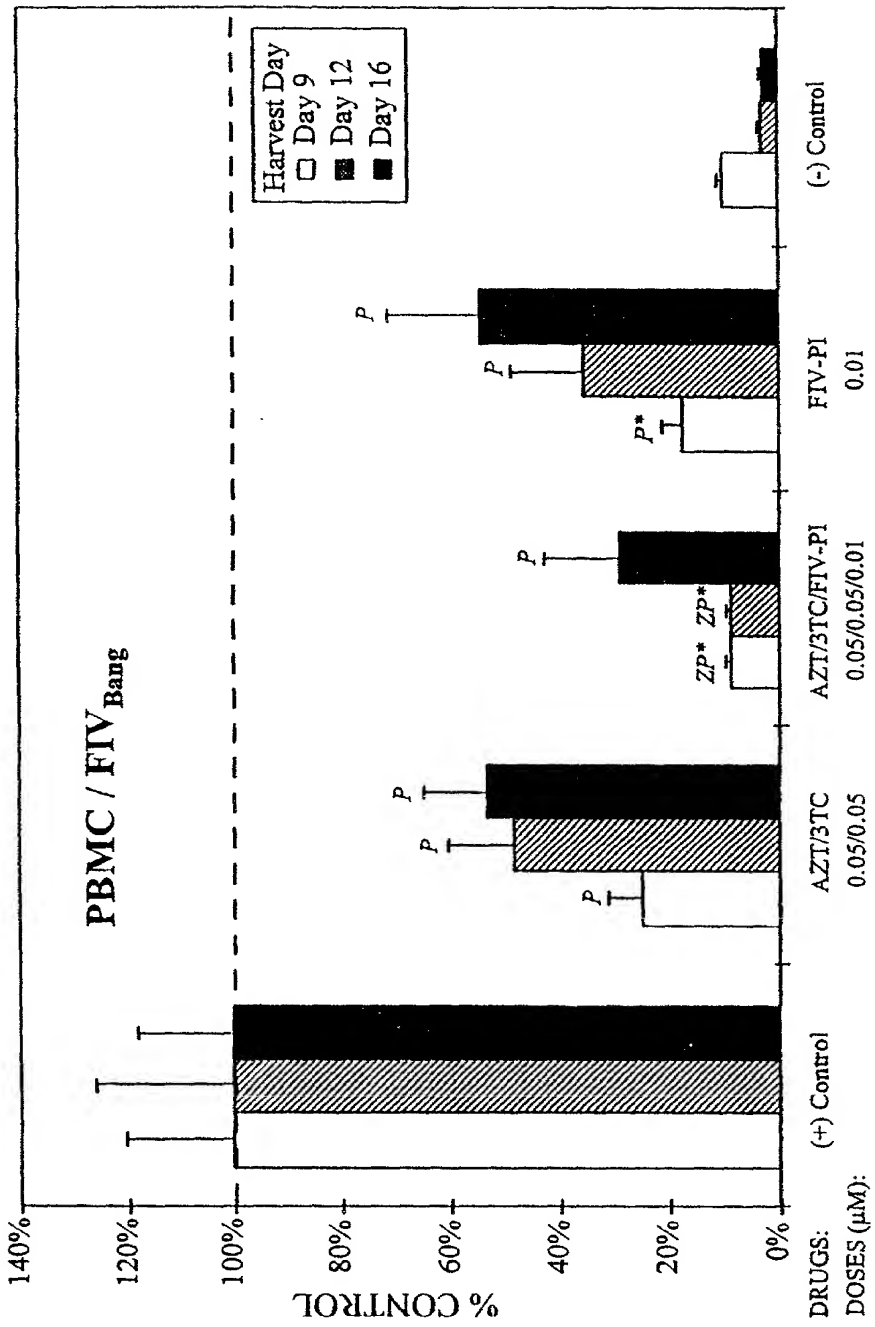
PBMC / FIV Bang
% CONTROL
140%
120%
100%
80%
60%
40%
20%
0%
Harvest Day
Day 9
Day 12
Day 16
P
P*
ZP*
DRUGS:
DOSES (μM):
(+) Control
AZT/3TC
0.05/0.05
AZT/3TC/FIV-PI
0.05/0.05/0.01
FIV-PI
0.01
(-) Control

FIG. 2B

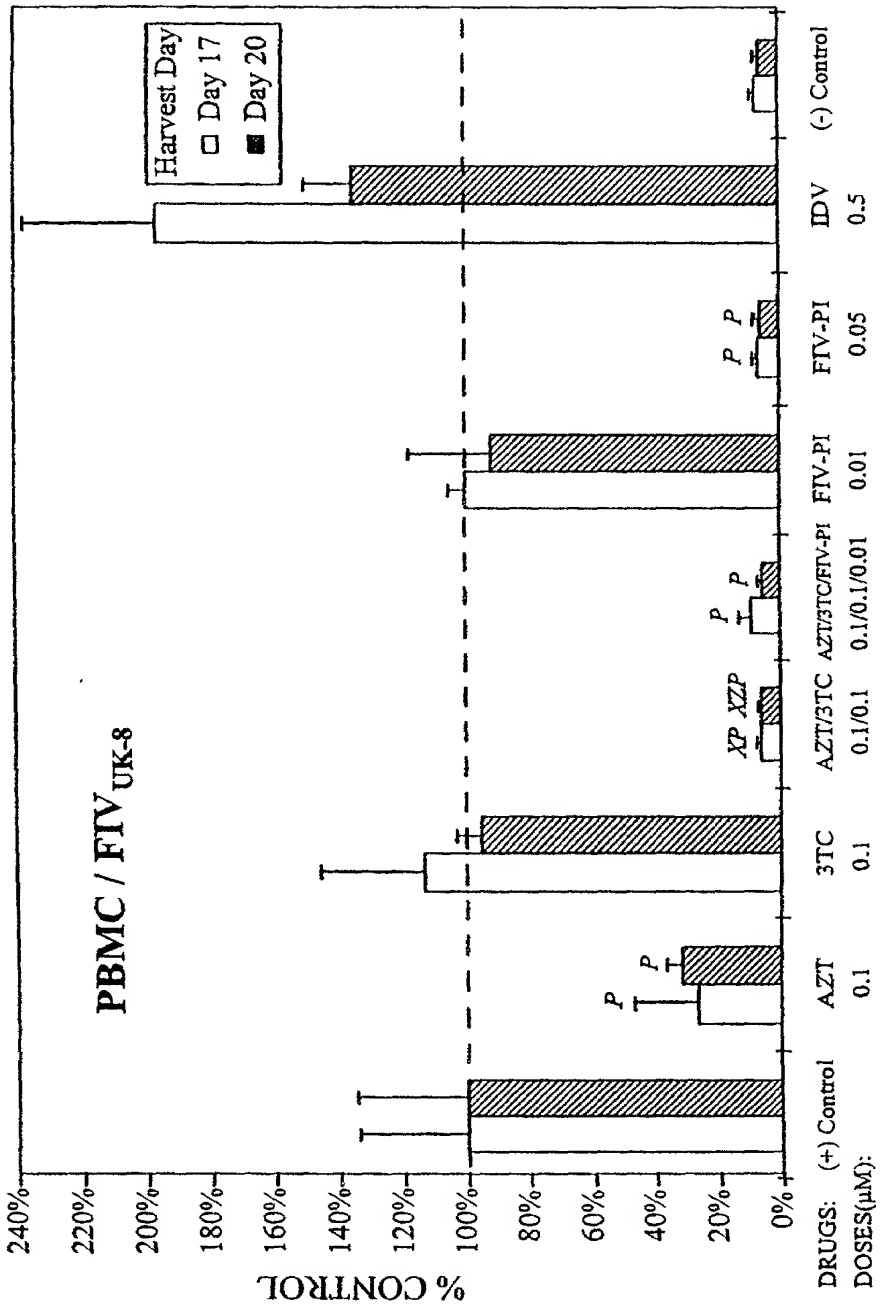
PBMC / FIV UK-8
% CONTROL
240%
220%
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
Harvest Day
Day 17
Day 20
P
XP XZP
DRUGS: (+) Control AZT 3TC AZT/3TC AZT/3TC/FIV-PI FIV-PI FIV-PI IDV (-) Control
DOSES(μM): 0.1 0.1 0.1/0.1 0.1/0.1/0.01 0.01 0.05 0.5

FIG. 3A

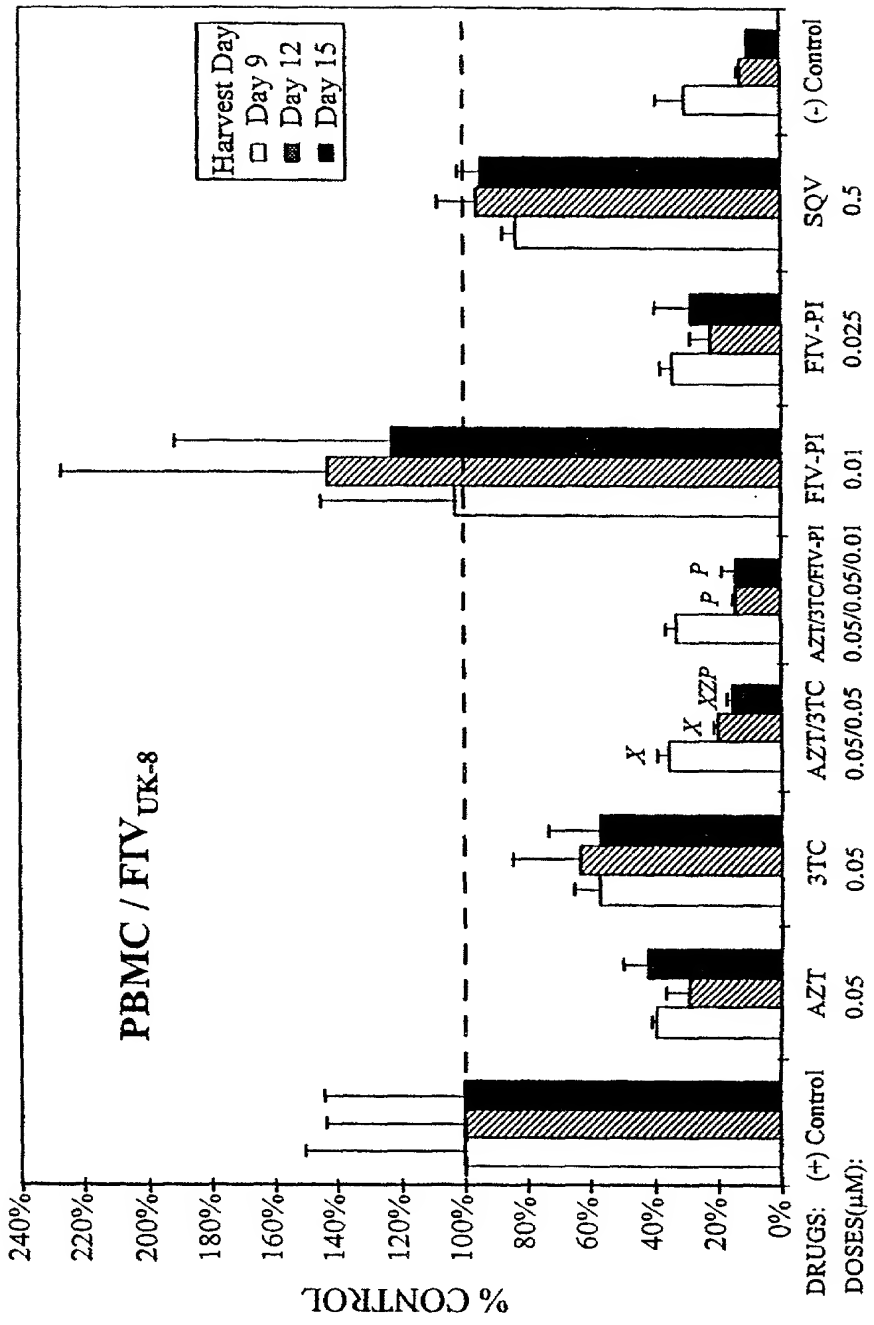
PBMC / FIV UK-8
% CONTROL
240%
220%
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
Harvest Day
Day 9
Day 12
Day 15
DRUGS: (+) Control AZT 3TC AZT/3TC AZT/3TC/FIV-PI FIV-PI FIV-PI SQV (-) Control
DOSES(μM): 0.05 0.05 0.05/0.05 0.05/0.05/0.01 0.01 0.025 0.5
X
XZP
P

FIG. 3B

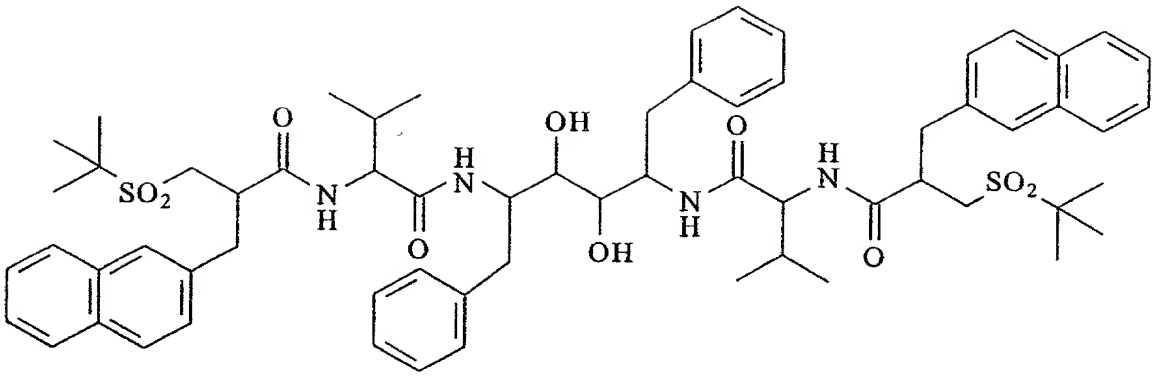

FIG. 4

Docket No. UF-219XC1

DECLARATION (37 CFR 1.63) AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence. post office address, and citizenship are as stated below next to my name; and

I believe that I am the original, first. and sole inventor (if only one name is listed below), or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **COMBINATION THERAPY FOR TREATMENT OF FIV INFECTION** the specification for which

☐ is attached hereto.

☒ was filed May 28, 1999, as PCT International Application No. PCT/US99/11940.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 and/or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Application Serial No.	Country	Filing Date	Priority Claimed

I hereby claim priority benefits under Title 35, United States Code §119 of any provisional application(s) for patent listed below:

Application Serial No.	Filing Date	Priority Claimed
60/087,281	May 29, 1998	Yes

I hereby claim the benefit under Title 35, United States Code, §120 and/or §365 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations. §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following persons registered to practice before the Patent and Trademark Office as my attorneys with full power of substitution and revocation to prosecute this application and all divisions and continuations thereof and to transact all business in the Patent and Trademark Office connected therewith: David R. Saliwanchik, Reg. No. 31,794; Jeff Lloyd, Reg. No. 35,589; Doran R. Pace, Reg. No. 38,261; Christine Q. McLeod, Reg. No. 36,213; Jay M. Sanders, Reg. No. 39,355; James S. Parker, Reg. No. 40,119; Jean Kyle, Reg. No. 36,987; Frank C. Eisenschenk, Reg. No. 45,332; Seth M. Blum, Reg. No. 45,489; Glenn P. Ladwig, Reg. No. 46,853.

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_____ Date _____

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